Chapter 11

Skeletal System

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The interaction between living things and the force of gravity has been a subject of scientific interest virtually since Newton formulated the law of gravity late in the 17th century. However, it was not until the late 19th and early 20th centuries that Tsiolkovskiy[1] and D'Arcy Thompson[2] named a definitive role for gravity in the link between the size of an animal and the mass of its skeleton (Tsiolkovskiy on theoretical grounds, and Thompson on the basis of a quantitative interpretation of Galileo's principle of similitude). Subsequent assessments of theoretical and experimental data by distinguished scientists such as Zenkevich (1944), Brovar (1960), Went (1968), Korzhuyev (1971), Maier (1974), Smith (1975), Shnol (1979), and others led to the conclusion that the evolution of land vertebrates involves progressive adaptation that acts to overcome gravitational force. The biomechanical role of the vertebrate musculoskeletal system is thought to depend on its sensitivity to gravity and changes in gravity.[3–5]

Research performed during the past four decades has led to replacing the old concept of bone as a static, supporting organ with the recognition that bone tissue is an active, multifunctional organ that has many functions beyond providing biomechanical support and protection. These functions include storing minerals, maintaining calcium homeostasis, and providing hemopoiesis within the bone marrow. The skeletal system not only supports the generation of new hemopoietic cells,[6] but also supports the continual differentiation of bone tissue itself.[7]

In all vertebrates, including humans, the growth, development, and involution of bone throughout life result from continual tissue remodeling. Bone remodeling is a complex process encompassing both systemic and local regulation.[8,9] Systemic control lies in neuroendocrine, hormonal, circulatory, and immune regulation; local control involves tissue, cellular, and intercellular interactions. The balance between bone resorption and formation tends to shift with age and mechanical challenges from the environment (e.g., gravity, types of motor activity, magnitude and nature of weight loads, etc.).[4]

The first systematic studies of the gravitational physiology of bone began in the early 1970s with animals aboard Kosmos biosatellites and under simulated-microgravity conditions. These U.S.-U.S.S.R collaborations were expanded to include human subjects in 1982 after new methods had been developed (e.g., computer tomography, mono- and dual-photon absorptiometry) that allowed skeletal status to be evaluated in vivo. A review of early results on reactions of the skeletal system in microgravity is presented in Ref. 10.

This chapter summarizes results collected regarding spaceflight-induced changes in bone mineralization in space crews, both from our own experience and from published reports in the literature. Special attention is paid to the use of modern techniques with which to analyze the dynamics and mechanisms of gravity-dependent changes in various parts of the skeleton. Of practical interest are the operational consequences of changes in mechanical force on bone during flight, and possible means of counteracting adverse effects on the human skeleton during return to gravitational environments. The chapter concludes with a brief overview of some remaining challenges for future research on this issue.

I. Human Calcium Balance and Mineral Density in Spaceflight

Living bone consists of an organic matrix of collagen fibers and noncollagen proteins or proteoglycans. This matrix is made rigid by minerals (hydroxyapatite and crystals of other calcium and phosphorus salts) incorporated in the collagen microfibrils and the interfibrillar space in the bone interstitium. Human bone tissue contains 98% of all the minerals in the body (calcium, Ca; phosphorus, P; magnesium, Mg; and sodium, Na), and 99% of the body's calcium.[11,12] The amount of calcium present per unit volume of bone can be used as an indicator of bone mass.[13]

The earliest demonstration that bone is lost in weightlessness was the finding that calcium balance had become negative after Gemini and Apollo missions.[14] Subsequent systematic studies of calcium metabolism during the three Skylab missions revealed consistent elevations in plasma concentra-

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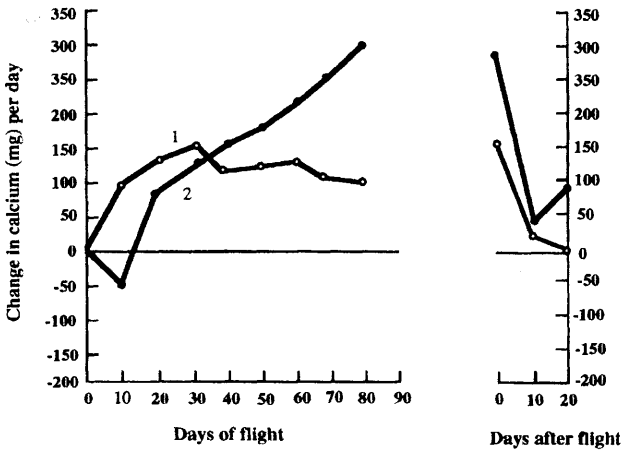

Fig. 1 Time course of calcium excretion in urine (open circles) and feces (closed circles) during and after Skylab flights lasting 28 (n=9), 59 (n=6), and 84 (n=3) days. From Ref. 16.

tions of calcium and phosphorus relative to preflight baselines.[15] The amount of excess calcium being excreted in the urine and the feces gradually increased with time spent in flight, and remained elevated until the end of the longest mission (Fig. 1). The mean rate of mineral loss was 0.6% of total body calcium per month, although this rate fluctuated substantially among individuals. The amount of calcium lost was weakly correlated with flight duration.[15,16]

Hypercalcemia also has been present in most cosmonauts after flights lasting 30 days or more.[17] The total calcium concentrations found in blood thus far have not exceeded physiological norms, but ionized calcium activity on flights lasting up to 237 days has exceeded preflight baselines by 16 to 32%.[18] This hypercalcemia was accompanied by negative calcium balance. Tests that involved ingesting calcium lactate and other substances led to the conclusion that the capacity of calcium-storage systems in the body was reduced after long spaceflights.[17,19]

Opportunities for more exact measurements were greatly enhanced by the development of noninvasive means of bone densitometry, including monophoton and dual-photon gamma-absorptiometry; quantitative computer tomography; quantitative digital (scanning) radiography or dual photon X-ray absorptiometry; neutron-activated analysis; and ultrasound[13] (Table 1).

The first evaluations of local mineral loss using X-ray photodensitometry revealed 2–17% decreases in the mineral density of the heel bones (calcaneus) and hands of Gemini and Apollo crews[20] and Soyuz-3 crews.[21] These losses were found later to have been exaggerated by methodological error.[22] Monophoton gamma-absorptiometry techniques later showed no appreciable change in mineral density of the calcaneus in Apollo-14 or -16 astronauts.[23] Decreases in calcaneal mineral density in the Skylab crews demonstrated with the same method differed considerably among individuals. One crew member lost 7.4% after the 59-day flight; two others lost 4.5% and 7.9% after the 84-day flight.[24] The mean monthly rate of decrease in this weight-bearing bone was greater than that of cortical bone tissue in the shoulder.[16,24]

Monophoton absorptiometry also revealed individual differences in calcaneal mineral density in eight Soviet cosmonauts who flew on Salyut-6 for 75 to 184 days. Decreases ranged from 0.9% after a 75-day flight to 19.8% after a 140-day flight.[10] Interestingly, losses after flights lasting 175 and 184 days were smaller (3.0 and 4.0%, respectively) than after the shorter flights, but in general the loss of mineral density was weakly correlated with the length of flight. The amounts of calcium excreted were greater than could be accounted for by the loss of mineral density in the calcaneus. Thus, no associations could be established between the magnitude of negative calcium balance and flight duration, or between calcium balance and exercise in flight.[16,17] The lack of correlation between the total calcium loss and the mineral density of the calcaneus prompted a search for other bones that could have been the source of the calcium loss.

From 1981 to 1987, seven cosmonauts were examined with computer tomography (General Electric model GECI-7800)[25] to quantify changes in mineral density of the compact and spongy (trabecular) bone in lumbar vertebrae after flights lasting 64 to 366 days. Results from four cosmonauts tested after 150-day or 211-day flights, and three after a 237-day flight, are shown in Table 2. Mineral density of the anterior and posterior aspects of the vertebrae were measured selectively, as were the density and volume of the iliocostal, sacrospinal, and interspinal muscles.[26] Four of the seven cosmonauts in this group had no changes in mineral density of trabecular bone in the center of the vertebral body, one had decreased density, and the remaining two had increased density. Density in the posterior elements of the vertebrae, which consist mainly of compact bone, decreased in four of the seven subjects by a mean of 7.8% ($p<0.01$). The volume of back muscles decreased by a mean of 4.4% ($p<0.01$), and their density decreased by 0.4% ($p<0.01$).[26,27]

During the 1990s, joint U.S.-Soviet investigations involved the use of a QDR-1000/W model dual-photon X-ray gamma-absorptiometer (manufactured by Hologic) to measure calcium mass in the entire skeleton and in the lumbar vertebrae, the hip, and the proximal regions of the tibia and femur[28] (Table 3). The average 0.4% loss of mineral from the entire skeleton was comparable to results from the Skylab calcium-balance studies.[16] Mineral density decreased significantly in the lumbar vertebrae, pelvis, and legs, which of course bear the most weight under normal 1-*g* conditions. The mean rates of mineral loss in different areas of the skeleton for cosmonauts on 4.5- to 6-month missions on Mir are presented in Fig. 2. Virtually all space crew members examined to date have shown moderate mineral-density loss in the lumbar vertebrae (5–10%), the femoral neck (1–11%), and the greater trochanter (up to 14%) during flight. Only two crew members had statistically significant decreases in mineral density in the tibial proximal epiphysis after landing.

Table 1 Noninvasive methods of measuring bone

Method	Body Part	Reproducibility, %	Precision, %	Time, min	Radiation, rem
Radiography	hand	2-3	10	1	1–2
Monophoton absorptiometry	ulna	2-3	< 2	10	5
	calcaneus	2-3	< 3	10	5
Quantitative computer tomography	spine	1-5	< 10	30	
Neutron-activated analysis	whole body	1-2	< 1	40	2
Dual-photon absorptiometry	spine	2-3	< 3	20	15
	thigh	2-3	< 3	20	15
	whole body	2	< 3	50	30
Dual-photon X-ray absorptiometry	spine	< 1	< 1	< 1	10
	thigh	< 1	< 1	10	< 1
	whole body	< 2	< 1	20	< 3

Table 2 Percent change in lumbar-vertebral density and back-muscle volume in seven cosmonauts after 150-, 211-, or 237-day spaceflights (Ref. 27)

	150-day flight		211-day flight		237-day flight		
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7
Mineral density							
Whole vertebra	–2.3	–10.8	–6.1	–0.3	–6.0	–7.0	–2.0
Vertebral body	+1.8	–10.0	–4.6	+2.9			
Vertebral processes	–7.5	+11.9	–8.1	–3.7			
Spongy tissue	+1.7	–10.2	+1.9	+12.4	–0.5	+2.0	+8.0
Back-muscle volume	–6.5	-5.8	–0.8	–4.3			

Units are percent change from preflight baselines measured by quantitative computer tomography.

Summary spaceflight data on the mean monthly rate of change in mineral density at specific locations are presented in Fig. 3. Although these results indicate that the greatest changes took place at or below the level of the lumbar vertebrae, considerable differences were apparent between individuals (Table 3). Group means for monthly mineral loss were 1% in the lumbar vertebrae (range 0.5–2.0%) and 1.8% in the greater trochanter (0.7–3.1%). The magnitude of these changes was not strongly correlated with flight duration. Moreover, some crew members had no changes at all in some areas (e.g., vertebrae, tibial diaphysis, or calcaneus), which may reflect variation among individuals or perhaps the use of countermeasures (e.g., exercise) during flight.

Information as to the recovery of mineral density after flight is scarce. Individual rates of recovery are extremely variable. Mineral density in the lumbar vertebrae of some cosmonauts had remained below preflight values as late as 10 and 18 months after landing—more than twice the duration of the flights themselves.

II. Animal Studies in Spaceflight and Simulations of Weightlessness

The use of animals in gravitational skeletal-physiology studies is advantageous for several reasons, first among them being the ability to study many structural and mechanical properties of bone tissue that cannot be studied in humans. Second, observational conditions can be optimized for animal experiments in space, and the confounding effects of countermeasure use can be eliminated. Third, varying the experimental conditions, studying several species, and comparing the results with those from humans all tend to confer a broader perspective on the problem.

Table 3 Percent change in bone-mineral density in the Mir prime crews 6–9 after 132- to 176-day flights (Ref. 28)

Subject	Flight duration, days	Head	Arms	Ribs	Thoracic Vertebrae	Lumbar Vertebrae	Pelvis	Femoral Neck	Greater Trochanter	Proximal Tibia	Legs	Whole Skeleton
1	132	+10.6	+2.2	−0.1	+2.9	−7.1	−6.0	−1.3	−8.1	−9.5	−5.1	−1.2
2	132	+3.6	+1.5	−3.0	+0.6	−7.9	−5.1	−6.0	−14.2	−0.8	−2.8	−0.5
3	145	+2.8	+7.8	−2.2	−2.9	−6.2	−3.0	−4.5	−2.0	−6.8	−0.4	+1.1
4	169	+0.9	−2.5	+0.8	−0.1	−5.5	−6.9	−3.5	−5.4	−0.4	−0.4	−0.6
5	169	−8.3	+0.2	+1.2	−9.5	−6.9	−7.7	−3.1	−9.0	−0.6	+0.9	−1.6
6	176	+2.9	−0.7	+2.1	−3.6	−10.1	−5.7	−11.4	−12.9	−0.7	−2.3	−0.6
7	176	+7.3	−1.5	+1.3	+3.6	+0.3	−4.5	−5.3	−5.7	−0.9	−1.3	+0.6
Group means		+2.8±2.2	+1.0±1.3	0.0±0.7	−1.3±1.7	−6.2±1.2	−5.6±0.6	−5.0±1.2	−8.2±1.6	+2.8±1.4	−1.6±0.7	−0.4±0.4

Units are percent change from preflight baselines measured by dual-photon X-ray absorptiometry.

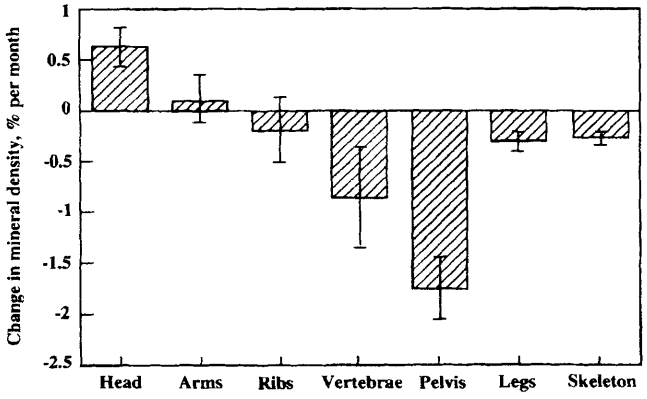

Fig. 2 Changes in regional mineral density, as measured by dual energy X-ray absorptiometry, of 11 cosmonauts after Mir missions lasting 5 to 6 months. From Refs. 27 and 28.

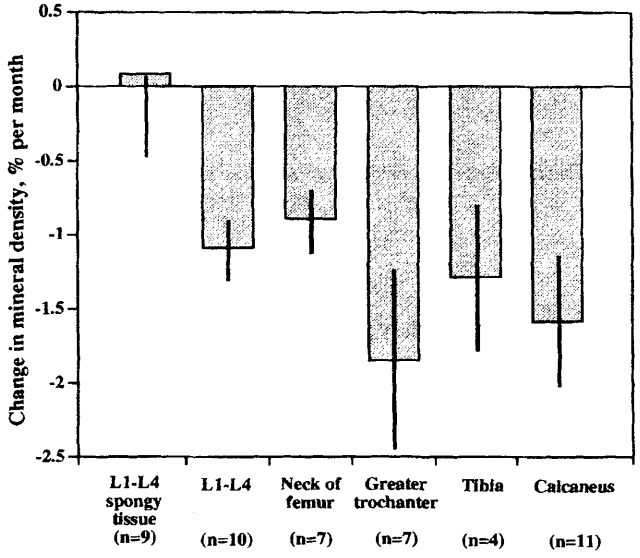

Fig. 3 Changes in local mineral density of cosmonauts after Mir missions lasting 3 to 6 months. Spongy tissue of the lumbar vertebrae was measured by quantitative computer tomography (Ref. 27), the calcaneus (heel bone) by monophoton absorptiometry (Ref. 10), and the other areas by dual energy X-ray absorptiometry (Ref. 28). Adapted from Ref. 27.

Space experiments with animals have been conducted since the 1970s on the Soviet Kosmos biosatellite series (Table 4). Participants in this series have included scientists from Russia, the United States, France, Bulgaria, Germany, Hungary, Poland, Romania, and Czechoslovakia. Animal experiments also have been flown on the U.S. Spacelab-3 (SL-3) and SLS-1 missions. The effects of weightlessness on the musculoskeletal apparatus of animals also can be simulated through immobilizing the animals, limiting their motion, or unloading their hind legs through suspension.[10,29,30] Suspension best reproduces the effects of weightlessness on bone[10,29] and muscles[31] and involves the smallest stress reaction of the three models.[32,33] Reviews of animal experiments can be found in Refs. 10, 34, and 35. The following paragraphs present a brief overview of flight results in the order of increasing mission duration.

Spaceflights as brief as one week have been shown to inhibit bone formation and reduce bone mass. The relative volume of the organic matrix (osteoid) in the proximal metaphyses of the femur and humerus in rats was reduced after the 5-day Kosmos-1514 mission.[34,36] Bone formation after 7-day flights on Kosmos-1667 and SL-3 was inhibited to different extents in different bones. The characteristic signs of osteopenia were noted in trabecular bone, which became thinner, with fewer and thinner trabeculae remaining; had lesser amounts of osteoid; and had fewer and less active osteoblasts.[34,37] Periosteal bone formation was slowed, and bone lengthening was less in the flight animals than in ground controls.[38] However, results relating the level of resorption with cellular activity are contradictory.[34,35,39]

Table 4 Chronology of animal experiments in space

Mission	Year	Flight duration, days
Kosmos–605	1973	22
Kosmos–690	1974	20.5
Kosmos–782	1975	19.5
Kosmos–936	1977	18.5
Kosmos–1129	1979	18.5
Kosmoa–1514	1983	5
Kosmos–1667	1985	7
Spacelab–3	1985	7
Kosmos–1887	1987	12.5
Kosmos–2044	1989	13.5
Spacelab–1	1991	9
Kosmos–2229	1992	13

For the SL-3 rats, mineralization of the osteoid was slowed as well as bone formation being depressed,[40] which suggests disruptions in both mineralization and collagen metabolism. These findings were confirmed by the appearance of type III collagen isoforms, which have lesser affinity for calcium than other isoforms,[41] in the femurs of rats aboard Kosmos-1514 and -1667.

Interestingly, the mechanical strength of the rat humeral proximal metaphysis after the Kosmos-1514 and Kosmos-1667 missions was less than that of vivarium control animals, despite the lack of change in the mineral density of this area in both groups.[36,42] Bones of the age-matched control group actually showed increases in bending strength and other biomechanical indices, but flight animals remained at preflight levels.[35] Overall, the results from these studies suggest that the bone becomes more brittle, even after flights lasting only a week, and cannot respond normally to loading and deformation.[36]

Rat-bone morphology and histochemistry after the 12-day Kosmos-1887 mission (when the animals were killed 48 hours after landing) have been interpreted as reflecting failures of modeling and remodeling.[34,43] Osteogenesis was inhibited in the femoral metaphyses and the tibial proximal epiphyses[44,45]; elevated reabsorptive activity was indicated by the presence of large Howship lacunae, increased osteoclast activity, structural rarefaction of spongy and cortical bone, disintegration of osteons, and signs of perilacunar osteolysis.[45]

The reduction of spongy tissue in the growth zones of the tibial proximal metaphyses[44] and the femoral distal epiphyses[46] found in this experiment was accompanied by reductions in both absolute numbers of osteoblasts and in the percentage of osteoblasts having high collagen-synthesizing activity. Other assessments of osteoblast production involving the maxillary periodontal ligament model have revealed that microgravity blocks a step in osteoblastogenesis in such a way as to increase the number of undifferentiated osteoblast progenitor cells and decrease the number of preosteoblasts.[46,47] Interference with collagen metabolism was evident as well from changes in the proliferative and differentiative behavior of chondrocytes in the tibial proximal diaphyses; the collagen fibrils were broader, and proteoglycan aggregates were of different sizes.[48] No significant changes were detected in the mineral content of the femur, tibia, humerus, mandibular bones, or lumbar vertebrae.[34,49]

The decrease in bone strength without change in mineral density described earlier for 5- to 7-day flights also was present in the humerus and femur of the Kosmos-1887 rats.[50] The lumbar vertebral bodies in the Kosmos-1887 rats also lost strength without changes being present in the amount or composition of minerals.[49] However, another experiment on the 13.5-day Kosmos-2044 flight (when the animals were killed 6–11 hours after landing) revealed no changes in bone structure, amount of osteoid, or bone strength.[51] Nor were changes noted in osteogenic cells[52] or collagen ultrastructure,[53] although some evidence of structural disorganization was present in the collagen-fibril network in cartilage.[54] The differences between the Kosmos-1887 and -2044 experiments could reflect differences in the ages of the rats (90 days on Kosmos-1887 and 110 days on Kosmos-2044), which could have affected skeletal sensitivity to weight loading, or the postflight delay of 48 hours before the Kosmos-1887 rats were killed. The presence of reactivated resorption noted in the Kosmos-1887 rats[45,50] tends to favor the latter possibility, since similar resorption patterns were detected in other animals killed on the second day after landing.

Flight studies with primates involving noninvasive bone densitometry have revealed histomorphometric indications of slowing in the rate of bone neogenesis[43] and decreased bone density[55] after 2 weeks of flight.

Periosteal growth the day after the 18- and 19-day Kosmos-782, -936, and -1129 missions was reduced by as much as 50% relative to control animals (Fig. 4).[56] Trabecular-bone volume in the tibia and humerus decreased by 30–35%, and the number of osteoblasts—but not osteoclasts—declined[57]; however, no signs of endosteal resorption were present in the compact bone. Cessation of growth was evident in the periosteal and endosteal areas.[56] Evidence from other studies suggests that osteogenesis had been blocked during flight, but recovered quickly thereafter.[58] Changes in the trabecular bone of the tibial proximal metaphysis may have been due to osteoclast resorption,[59] but resorption in the femoral proximal epiphysis and tibial distal epiphysis was thought to reflect perilacunar (osteocytic) osteolysis.[60]

No significant changes were detected in the compact bone of the femoral diaphyses[10] or in the spongy tissue of the vertebrae after nearly three weeks of flight (Kosmos-782, -936, and -1129). However, some authors[34] have interpreted results from the Kosmos-1129 experiment as indicating changes in the quality and delays in the maturation of collagen, which is consistent with results from others on collagen microstructure in space.[41,51,53] Mean losses of mineral density in the femoral distal epiphysis in the Kosmos-605, -936, and -1129 groups ranged from 7.5 to 21%; mean losses in the femoral head ranged from 5.2 to 17.7%.[10] The amount of calcium in the

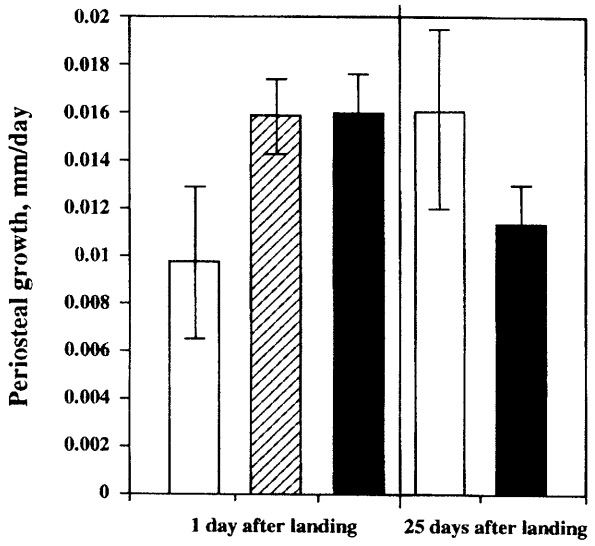

Fig. 4 Periosteal growth in the tibia of rats 1 and 25 days after the 19.5-day Kosmos-782 mission (white bar) relative to that in synchronous control rats (hatched bar), and vivarium control rats (black bar). Growth in the flight group was significantly different from both controls at both measurement periods, $p<0.05$.

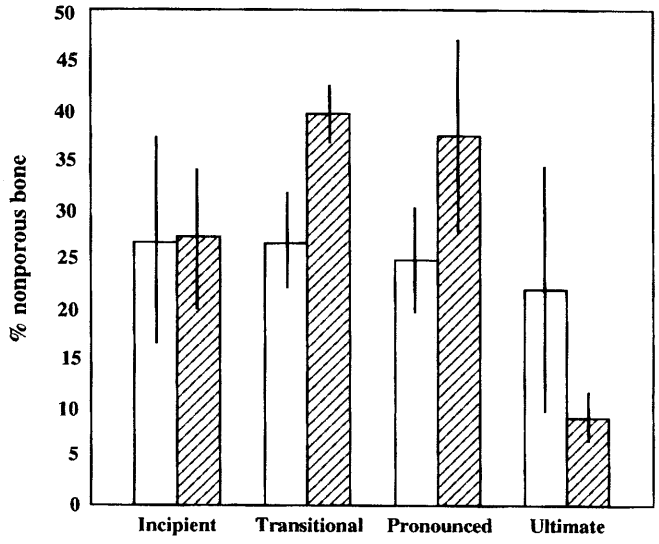

Fig. 5 Degree of mineralization, measured by micro X-ray differentiation, of the tibial diaphysis in rats after the 19.5-day Kosmos-782 mission. White bars indicate the flight group and hatched bars the vivarium-control group. From Ref. 59.

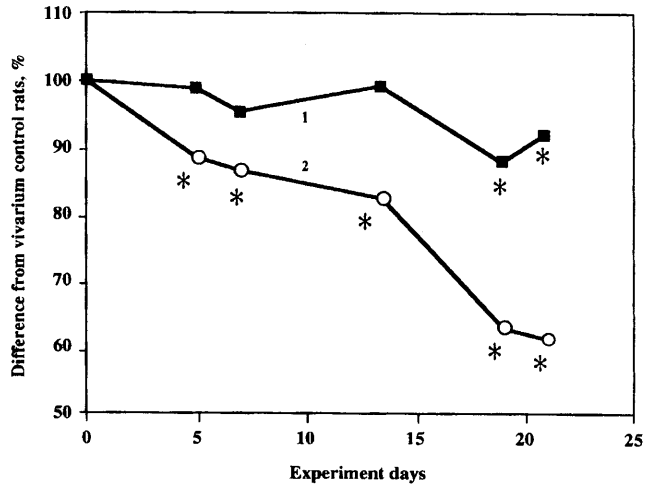

Fig. 6 Changes in mineral density (squares) and strength (circles) of the humeral and femoral heads of rats on Kosmos biosatellite missions 1514, 1667, 2044, 782, and 605. *Significantly different from vivarium control rats, $p < 0.01$. Adapted from Refs. 10, 36, and 42.

ash residue was less than that in control animals, and the ratio of calcium to phosphate was reduced as well. Micro X-rays revealed loci of both hyper- and hypomineralization in the cortical layer of the tibia (Fig. 5), perhaps indicating incomplete bone resorption.[59] In the vertebrae, calcium content was reduced, but areas of concentrated mineralization were present in the cortical and trabecular tissue.[61] Similar patterns of mineral loss and accumulation were present in the femurs of rats after a 1-week flight on SL-3.[62] These data suggest that bone mineralization and maturation of new bone are slowed, and provide another piece of evidence to indicate that bone remodeling slows in weightlessness. The changes noted in bone resorption also could favor the preservation of old, highly mineralized bone tissue.

Biomechanical assessments of rat bones after 3-week spaceflights revealed decreases in the bending strength of trabecular bone and in mineral density (Fig. 6).[10] The decreases in breaking strength (20–30% in different experiments) may be more pronounced than would be apparent from the 15–30% decreases in the coefficient of elasticity. Changes in both indicators were greater in the femoral head than in the humerus. An unexpected increase in breaking strength of the femoral-diaphyseal compact bone[10] was thought to result from increased mineralization, as described for the cortical bone in both the tibia[59] and the femur.[62] Bending tests with whole tibias or femurs revealed declines in their structural resistance, and fractures were noted in the proximal and distal metaphyses. This latter finding is not unexpected, since these areas contain trabecular bone from which tissue presumably had been lost.[10,50]

Finally, the early readaptation period after 19 days of flight[10] or 21 days of suspension[50] has been associated with further decreases in bone strength, even as the calcium level and total mineral density should begin to recover. The first 3–4 days after flight may involve activation of bone resorption from reexposure to normal mechanical stimulation,[50,60] while bone formation remains suppressed.[35]

III. Human Bone Responses in Simulations of Weightlessness

Both horizontal and head-down bed rest are common means of mimicking the skeletal response to physical "unloading."

Research conducted with healthy volunteers confined to bed, although not a perfect imitation of spaceflight conditions, has allowed closer, more detailed observations than are possible with space crews. More subjects are available; more questions can be asked and more hypotheses tested; and experimental conditions can be controlled better in ground-based experiments than in flight.

Long periods of bed rest, in which weight bearing is removed from the skeleton along the Z-axis of the body, is well known to produce "thinning" in X-ray patterns of bones, otherwise known as disuse or immobilization osteoporosis.[63] In clinical medicine, the term osteopenia, which describes a reduction in bone density or calcification,[64] has supplanted the term osteoporosis, which refers to diseases of diverse etiology that cause a reduction in bone mass per unit volume, but do not affect the mineral:organic ratio. However, in this chapter, the term osteoporosis is used to refer to a particular form of osteopenia in which an individual has less bone tissue than is appropriate for his or her age, with no known precipitating condition. According to this definition, the distinguishing feature of osteoporosis is the presence of at least one fracture.[64–66]

The experiments described below were designed to elucidate the mechanisms that underlie the development of "hypogravity osteopenia," to determine its rate of onset and development; to determine the magnitude of bone loss; and to test potential means of preventing or correcting functional (biomechanical) insufficiency of the skeleton.

A. Calcium Balance

According to the data presented in Table 5, "pure" hypokinesia (i.e., bed rest without the use of prophylactic interventions) produces calcium loss of 0.5% per month,[24,67–69] which is comparable to the magnitude of the negative calcium balance observed in the Skylab astronauts beginning in the second month of flight.[15] By way of comparison, subjects who wore casts on their lower torsos lost 1–2% of their total body calcium during a 6-week period[70]; patients with poliomyelitis lost less calcium than these casted subjects, but still lost more than did healthy subjects confined to bed.[71]

Our previous attempts to correct the severity and rate of calcium loss were unsuccessful, despite the use of active (physical exercise) and passive (mechanical) countermeasures.[72] However, some countermeasures (e.g., exercise combined with biphosphonates or vitamin supplements) can hold the amount of calcium lost to about half that of the astronauts.[69,73] The point at which countermeasure protocols are begun during bed rest is crucial in preventing or slowing calcium loss. In a recent 370-day study of head-down bed rest,[73] the magnitude of negative calcium balance in a group of volunteers who exercised continually during bed rest was half that of a group that began countermeasures on the 120th day of the experiment (Table 5). The development of negative calcium balance in these groups over time is illustrated in Fig. 7. The group that began exercising after the first 120 days of bed rest continued to lose calcium, and apparently began to recover only during the final third of the bed-rest period.[73]

B. Regional Losses of Mineral Density

The results illustrated in Fig. 3 suggest that the location of bones with respect to the normal gravity vector influences the reaction of those bones to a change in or loss of weight loading, whether from bed rest or spaceflight. For example, mineral loss in the calcaneus would be greater than that in the femur, which in turn would be greater than that in the lumbar vertebrae. In addition to location, the tissue properties (e.g., trabecular vs compact bone) and the biomechanical functions of bones also influence their reaction to unloading. For example, loss of mineral density in the trabecular portion of the lumbar vertebrae after spaceflight was greatest in the lower vertebrae, which bear the greater load on Earth (Fig. 8). In contrast, losses of mineral density were comparable among the L1–L3 vertebrae in two subjects after a 370-day head-down bed-rest period (Fig. 9),[74] which may reflect the presence of the gravitational vector in this model of weightlessness. Finally, as alluded to in the previous section on calcium balance, many organ systems (cardiovascular, muscular, renal, gastrointestinal, and others) participate in the regulation of mineral transport, and thus in bone formation. The sections below describe changes in the mineral density of the calcaneus, the long leg bones, and the lumbar spine in response to bed rest.

1. Calcaneus and Other Foot Bones

Bed rest induces decreases in the mineral density of the calcaneus (Table 5) and other bones of the foot. The mean monthly rate of mineral loss has varied in different experiments, but ranges from 2.8%–5% (roughly twice that of astronauts and cosmonauts[10,16,24]) and seems to depend on the age of the subjects and the use of countermeasures.[10,69,75,76] Even this is not clear, though, as different investigations have involved different countermeasure protocols and different density-measurement techniques. For example, in one study,[76] exercise (walking, running, or jumping) during the bed-rest period held the loss of bone mineral to half that reported elsewhere; however, other studies have shown exercise to have little or no effect on maintaining calcaneal density.[69,72] Exercise seems to be more effective in combination with drugs (Table 5), but drugs alone also can have a protective effect.[69,75] The time at which countermeasure protocols are begun affects the outcome for calcaneus density.[73] Even subjects who exercised throughout a 370-day bed-rest experiment had lost calcaneal density by the 120th day of bed rest; however, density in this group remained at that 120-day level throughout the remainder of the experiment. The group that began exercise on the 120th day of bed rest also had lost calcaneus density by that time, but continued to lose density throughout the remainder of the bed-rest period. This result contrasts with the calcium-balance results presented in Table 5 and Fig.

Table 5 Changes in calcium balance and regional mineral density in healthy humans during bed rest

Test	Condition	Treatment Duration, days	No. of Subjects	Ages of Subjects, years	Total Calcium Loss, g	Rate of Calcium Loss, g/mo or [%]	Mean Δ in Calcaneus Density, %/mo	Densitometric Method, Calcaneus	Δ in Lumbar Vertebral Density, Range or %/mo	Densitometric Method, Vertebrae	Reference
1	BR (no countermeasures)	28	44			[0.5]					67
2	BR (no countermeasures)	28	6	20±0.3			−5.05	MPA			10
3	HDBR, −6°	28	19						−7 to +10	DPA (L2-L4)	77
4	BR	35	6						−2 to 0	DPA (L2-L4)	78
5	BR	120	6	34.5±2.2			−3.5	MPA			10
6	BR	120	14	20–44				MPA			75
	•BR (no countermeasures)	120	7				−2.8	MPA	−1.72	QCT (L1-L2)	75
	•BR+drugs	120	7				−1.4	MPA	+0.91	QCT (L1-L2)	75
7	BR	120	6	19–52					−0.23±0.004	DPA,DXA (L2-L4)	78
8	HDBR, −4°, −5°	120	14	25–44	10–35	5.6 [0.47]					68
9	HDBR, −5°	120	15	26–44				NAA		QCT (L1-L3)	69
	•HDBR (no countermeasures)		3		14–35	6.1 [0.5]	−3.8	NAA	+3.15	QCT (L1-L3)	69
	•HDBR+exercise		4		7–14	2.8 [0.23]	−4.0	NAA	+1.05	QCT (L1-L3)	69
	•HDBR+drugs		4		3–18	1.3 [0.1]	+0.8	NAA	−0.20	QCT (L1-L3)	69
	•HDBR+exercise+drugs		4		5–18	2.9 [0.24]	−3.0	NAA	+0.60	QCT (L1-L3)	69
10	BR	182	12					π-MS			76
	•BR (no countermeasures)	182	6				−2.8	π-MS			76
	•BR+exercise	182	6				−1.6	π-MS			76
11	BR	42–252	90			6.0 [0.5]	−3.9	MPA			72
12	BR (no countermeasures)	168–252	15			[0.5]					24
13	HDBR, −5°	370	9	27–41							73,74
	•HDBR+countermeasures wks 1-53		4		24–41	2.67 [0.25]	−0.93	MPA	−1.0 to +0.4[a]	QCT, DPA (L2-L4)	73,74
									−0.4 to +1.1[b]		73,74
	•HDBR+countermeasures wks 18-53		5		60–89	6.34 [0.5]	−0.82	MPA	−2.4 to +1.6[a]		73,74
									−0.7 to +0.6[b]		73,74

BR, horizontal bed rest; HDBR, head-down bed rest; MPA, monophoton absorptiometry; NAA, neutron-activated analysis; π-MS, π-meson screening (Ref. 33); DPA, dual-photon absorptiometry; QCT, quantitative computer tomography; DXA, dual-photon X-ray absorptiometry. [a]range of change in spongy (trabecular) bone; [b]range of change in whole vertebrae

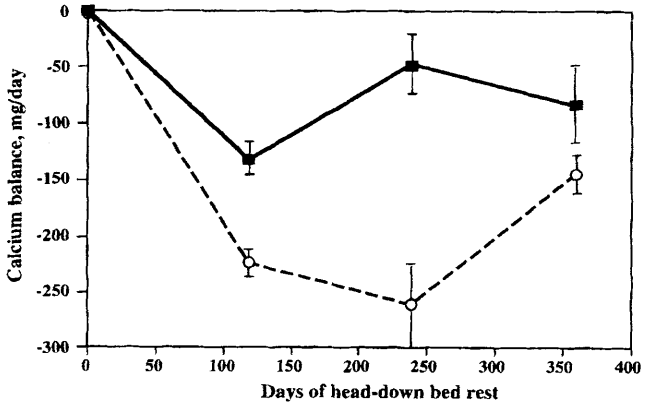

Fig. 7 Calcium balance in nine men during a 370-day period of head-down (–6°) bed rest. Four men exercised and took drugs to prevent bone loss throughout the bed-rest period (squares); five took no drugs and exercised only after the first 120 days (circles). Adapted (with permission) from Ref. 73.

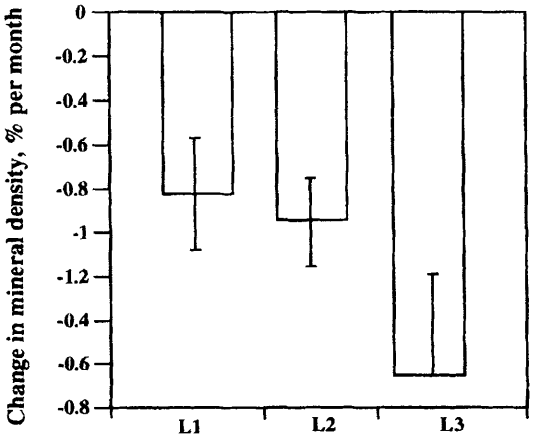

Fig. 8 Changes in mineral density, as measured by dual energy X-ray absorptiometry, of lumbar vertebrae in nine cosmonauts after 4.5- to 6-month Mir missions. From Ref. 28.

7. However, the wide variability among subjects in the 370-day study, which resembles that reported elsewhere, underscores the need for caution in interpreting these findings.

2. Leg Bones

Total mineral density of the tibia and fibula was measured with quantitative computer tomography between the mid- and distal third of the calf in healthy subjects (aged 25–44) who underwent 120 days of –5° head-down bed rest.[69] One of the three subjects in the control (no countermeasures) group showed an 8% decline in mineral density; 5 of the 12 subjects in the other three groups (exercise only, drugs only, or exercise plus drugs) displayed decreases ranging from 4.2–5.4%. Although changes in group means were not statistically significant, density tended to decrease in the exercise or drug groups, and tended to increase in the exercise+drugs group.

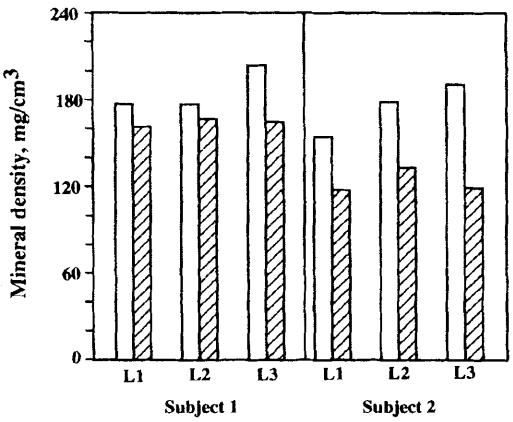

Fig. 9 Changes in density of spongy tissue of the lumbar vertebrae (measured by quantitative computer tomography) in two men after 370 days of –6° head-down bed rest. White bars, before bed rest; hatched bars, after bed rest. From Ref. 74.

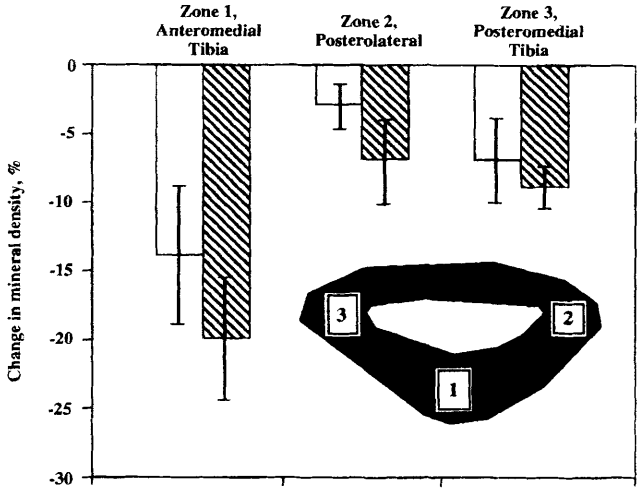

Fig. 10 Changes in mineral density of three tibial zones in nine men during a 370-day period of head-down (–6°) bed rest. White bars represent results from four men who exercised and took drugs to prevent bone loss throughout the bed-rest period; hatched bars represent results from five men who took no drugs and exercised only after the first 120 days. Adapted (with permission) from Ref. 73.

Tibial density measured by monophoton densitometry was decreased by 10% in three of four members of another group who underwent 370 days of head-down bed rest while exercising throughout.[74] Tibial density decreased by 5–12% in five other subjects in this study who exercised only during the 18th through 53rd weeks of the bed-rest period.[74] Group means were not significantly different from one another, but density in the group that began exercising on week 18 seemed to decrease during the second month of bed rest, i.e., 2 months earlier than in the continuous-countermeasure group.[74] However, computer tomography of the anteromedial zone of the mid- to lower tibial diaphysis revealed substantial fluctuations ranging from 5–28% of baseline in that area (Fig. 10). Both

groups, i.e., those who exercised throughout the bed-rest period and those who exercised only during the latter half, lost the most density in this zone, which is subject to large strain and dynamic load during locomotion.[11]

Dual-photon absorptiometry indicated that femoral-diaphyseal density increased toward the end of the bed-rest period.[74] Conversely, density of the femoral neck, where trabecular bone predominates, was reduced in both groups, although to a statistically significant extent ($p<0.01$) only in the delayed-countermeasure group. Decreases for some individuals in both groups reached 18 to 21%. Overall, the amount of mineral loss in the femoral proximal epiphysis was not different for the two groups.

3. Lumbar Spine

The patterns of change in mineral density in the lumbar vertebrae during bed rest (summarized in Table 5) are complex. Density in these bones did not change in all subjects; the probability of change seemed to increase with the duration of the bed-rest period. For example, only 2 of 19 subjects in a 30-day, –6° head-down bed-rest study showed significant changes in the L2–L4 region (one lost 7%, and the other gained 10%).[77] In another study, quantitative computer tomography revealed losses of mineral density in the L2–L4 region for six subjects (mean 1.7% per month) after 17 weeks of horizontal bed rest; density for another six subjects, who took drugs during the bed-rest period, tended to increase.[75] Dual X-ray absorptiometry used in another 17-week study revealed a 3.9% decrease (1% per month) in density of the lumbar spine for six subjects.[78] In yet another 17-week study, this one involving –5° head-down tilt, mineral density in the L1–L3 region increased for 6 of 13 subjects who did not use countermeasures, and also increased for 4 of 15 subjects who did use various protective and prophylactic procedures.[69,74]

Changes in the density of the trabecular portion of the lumbar vertebrae after either horizontal or head-down bed rest resemble those detected after spaceflight.[26] Isotope transmission absorptiometry and computer tomography both have revealed gains or losses in trabecular bone in the vertebral body; the changes noted with quantitative computer tomography, however, may reflect measurement artifact.[27]

Individual differences in the response to bed rest, which are appreciable even during relatively brief periods, become pronounced as the hypokinetic period lengthens.[74] For example, quantitative computer tomography revealed 12% and 30% decreases in lumbar trabecular bone in two subjects after 370 days of head-down bed rest; four other subjects showed *increases* ranging from 11–27%. Dual-photon absorptiometric measurements every eight weeks revealed slight increases in lumbar-vertebral density in the group that used countermeasures throughout the bed-rest period; the delayed-countermeasures group showed an analogous tendency.[74] In fact, the tendency for mineral density to increase seems to be more pronounced for subjects who do *not* use countermeasures.[69] This phenomenon deserves further study.

Finally, as is the case for spaceflight, mineralization tends to increase in the upper portions of the skeleton, especially the skull, in head-down bed rest.[78] The association between the severity of bone loss and the 1-*g* biomechanical function of the skeleton is noteworthy: The absolute loss of bone mineral seems to increase as one moves in the direction of the gravity vector. However, this result also could reflect errors in the measurement technique, and should be interpreted cautiously.

C. Hypermineralization in the Lumbar Vertebrae

The value of preserving mineral density is plain; however, the presence of hypermineralization in approximately one-third of the subjects undergoing either spaceflight or bed rest[26,27] requires additional analysis. Hypermineralization has been detected in people confined to bed with scoliosis[79] or spinal-cord injuries.[80] We first identified this phenomenon in healthy volunteers in association with 120 days of head-down bed rest.[69] We have since found mineral density to increase in the lumbar vertebrae both with quantitative computer tomography[26] and with transmission absorptiometry.[69,74] This phenomenon is much more prevalent in head-down bed rest than in horizontal bed rest, and thus probably is related to the complex circulatory reaction to the head-down position (see Chapters 3, 4, and 13 for further details). Hypermineralization during bed rest is associated negatively with physical exercise and positively with the use of biphosphonate drugs. Our assessments of possible reasons for the hypermineralization findings are given below.

As noted earlier, these findings could reflect errors in the measurement technique, errors in the correction factors used to estimate amounts of bone marrow and fat, or artifacts of changes in amounts of fatty tissue resulting from the lack of physical activity with bed rest. All densitometric methods depend to some extent on the accuracy of fat-mass estimates.[81] We ourselves have noted changes in fat mass after spaceflight with dual X-ray absorptiometry (unpublished data).

This phenomenon also could reflect an increase in intradisk pressure that is inappropriate for the weightless environment. Hyperhydration of the intervertebral disks could create additional loads on trabecular tissue of the vertebrae, transmitted through the end plates and affecting mineralization.[82] Magnetic-resonance imaging (MRI) verified enlargement (thought to be synonymous with hyperhydration) of disks during a 120-day period of horizontal bed rest, but mineral density was lost in these subjects.[83] Intervertebral disk pressure in the lumbar region of rats after the 2-week Kosmos-1887 flight was no different from that of a control group.[84] Some signs of dehydration of the fibrous ring and decreases in lumbar-disk mass were present in animals flown on Kosmos-2044.[85] From the available data, then, intervertebral disks may get bigger in space, but no evidence exists to suggest that hyperhydration or increased disk pressure causes increases in bone density.

The hypermineralization phenomenon also could be related to changes in torsional loading of the muscles surrounding the

spine.[69] Theoretically, in weightlessness, the predominance of tensile and torsional stresses over compressive loading (lack of weight and maintenance of muscle force) could lead to increased osteocyte activity and thus increase the size of the osseous lacunae.[86] If these lacunae fill with collagen-like substance synthesized by the osteocytes and become perilacunar bone, then that might account for the increase in mineralization.[64,86] Also, slight changes in spinal curvature due to different patterns of muscle force in weightlessness could realign the vertebrae, which would shift the angle of measurement and inflate estimates of the amount of mineral present per unit area.

A true increase in bone mineral would be reflected by sclerosis or the accumulation of mineralized osteoid from slowed or reduced bone remodeling in the absence of mechanical load. This supposition was confirmed in rats that had flown on 3-week spaceflights, in which mineralized osteoid had accumulated in vertebrae[61] and long leg bones.[10,59,62] Biomechanical tests of the long-bone epiphyses showed decreases in maximum strain concurrent with transient increases in the coefficient of elasticity.[50] Additional evidence for this supposition was provided from the bones of cosmonauts who died returning from a 28-day spaceflight.[87] Mineralization of the trabecular bone in the vertebral bodies had increased somewhat, although numerous microfractures also were present. This finding also implies that the bone-remodeling process, including resorption, is suppressed, which could explain the retention of traces of earlier microfractures and old areas of mineralized bone in these samples.

D. Morphological Changes

Studies of structural change in human bone resulting from lack of mechanical load have relied primarily on the use of biopsy specimens from the iliac crest region of subjects undergoing bed rest. This region has been deemed appropriate both for theoretical reasons[88] and because mineral density seems to change selectively in different bones in the pelvic region after spaceflights.[28]

Although data are somewhat scarce, results from biopsy studies of human bone are compatible with results of animal studies in weightlessness. As early as 7 days into a –6° head-down bed-rest period, seven of eight men had a mean 22% decrease in bone formation and a mean 7% decrease in osteoid mineralization.[88] No evidence of activated bone resorption was found; in fact, the number of osteoclasts (per unit area of tissue surface) declined, although the total resorption surface was comparable to that of an ambulatory control group.[88]

Biopsy data also were obtained from three subjects who did not use countermeasures during a 120-day head-down bed-rest study.[69] In these subjects, trabecular mineralization was reduced by 12–36%, and cortical mineralization by 15–35%; trabecular density (per unit volume) was decreased, the distance between trabeculae was increased, and osteoclast activity may have increased. However, no bone mass was lost.[89] Despite the presence of a negative calcium balance, mineral density increased by a statistically significant 12.6% in the lumbar vertebral bodies.[69] Another group in this same 120-day study, one that exercised during the bed-rest period, showed no histomorphometric evidence of change in bone formation, but bone resorption increased.[89] Mineral density of the vertebral bodies was no different from control measurements collected before the bed-rest period.[69] A third group, who were given drugs as countermeasures, had suppressed bone formation and resorption,[89] but no change in the lumbar-vertebral density and statistically insignificant negative calcium balance.[69] Histomorphometric changes in a fourth group, who both exercised and took drugs, were analogous to those in the third (drug-only) group.[89] The mineral density of the lumbar vertebrae did not change, but calcium balance became negative, although to roughly half the extent of the no-countermeasures group.[69]

These results suggest that physical exercise seems to activate resorption and prevent increases in mineral density in trabecular bone during bed rest. This supposition is consistent with another postulate that the increased mineralization in the no-countermeasures group was secondary to suppressed resorption in the absence of mechanical load. The inhibitory effect of the drugs used in this study on bone resorption, and the concurrent diminishment of bone formation, may help to re-establish normal calcium balance. The reason why the combination of exercise and drugs during bed rest was less than optimal in preventing negative calcium balance is unknown.

In another study,[73] no significant changes in bone formation or resorption were found in nine subjects after 120 days of head-down bed rest.[90] Neither the width of the iliac-crest cortex or the total volume of trabecular bone changed; however, fewer trabeculae were present, and those that were tended to be broader than control measurements.[90] The hypothesis that this effect resulted from activated remodeling, begun early during bed rest,[90] is difficult to reconcile with previously described findings of changes becoming evident after only 7 days of bed rest.[88]

However, the nine subjects described above went on to complete another 250 days of head-down bed rest. Of those nine subjects, five did not exercise during the first 120 days of the study and then began to do so for the remainder of the experiment.[73] During the first 120 days, those five subjects showed both earlier and greater losses of mineral density in the femoral neck[74] and a significantly more negative calcium balance than the other four subjects, who exercised throughout the study period.[73] Moreover, the biopsy material from the first group of five had suffered significant biomechanical degradation (e.g., detrimental changes in breaking load, rigidity, and crushing energy) at the 120th day of bed rest. Partial recovery was noted in another biopsy sample taken at the end of the 370-day bed-rest period.[91]

A caveat is needed here in order to interpret these results: Normally, histological results from iliac-crest bone in healthy bed-rested individuals correspond poorly with biomechanical results. Nor do these histological results correlate well with

densitometry of the lumbar vertebrae (despite the proximity of these bones), nor with balance studies. Thus, interpreting histomorphometric results can be quite difficult.[35,90] These disparities could be related to local differences in the reactions of bones in different areas of the skeleton. For example, healthy subjects exposed to weightlessness or bed rest show unequal shifts in mineral density throughout the tibia, and the usual gradient of mineral density in the L1–L4 vertebrae tends to become level. These observations underscore the importance of "unloading" in the development of local osteopenia.

IV. Regulation of Calcium Metabolism

Diet, renal and intestinal function, central and peripheral hemodynamics, and many other factors are known to influence the direct effects of mechanical and gravitational stimuli on bone structure. The interactions among these factors are coordinated through a complex hierarchy that ultimately balances the concentration of calcium between blood and bone and regulates the adaptation of bone to mechanical loads as well. Both local and systemic factors participate in this complex system. Local factors include oligopeptides, prostaglandins, and growth factors synthesized directly by bone cells, and proteins of the bone matrix such as osteocalcin, fibronectin, osteonectin, osteopontin, and others.[9,92] Systemic mechanisms represent neuroendocrine, hormonal, circulatory, metabolic, and immune control of calcium metabolism, and are reviewed briefly below.

Hormonal regulation of calcium metabolism during spaceflight has been discussed elsewhere,[10,17,93] but consensus has yet to be achieved. Blood concentrations of parathyroid hormone (PTH) and calcitonin were unchanged after 7 days of head-down bed rest[88] or spaceflight.[77,94] A transitory increase was noted in 1,25-dihydroxy-vitamin-D_3 after these spaceflights, but not after the 7-day bed-rest period.

Periodic fluctuations of PTH were noted during the 28-, 59-, and 84-day Skylab flights,[16] although blood concentrations of minerals and hormones remained within normal limits during the 28-day Skylab-2 mission.[35] Unpublished analyses of calcium and bone-matrix degradation products in urine samples suggest the presence of bone resorption and bone loss during these missions. Lumbar-vertebral density[26] was not correlated with blood concentrations of calcium-regulating hormones[94] in cosmonauts after 150- to 237-day flights. Blood concentrations of PTH, calcitonin, and vitamin D metabolites were unchanged, or remained within normal limits, for these cosmonauts and for subjects in a bed-rest study.[95] These results could reflect shifts in the sensitivity of the target organs to the relevant hormones,[88,96] or possibly one or more local causes.

Systemic regulation of the mineralotropic hormones is not well understood because of the complexity of the interactions between these hormones and those associated with physical and emotional stress, fluid and electrolyte regulation, and others.[93] For example, diminished bone formation is strongly correlated with high amounts of blood glucocorticoids.[35,93] However, other factors influence calcium homeostasis as well, chief among them being the cascade of effects resulting from headward fluid redistribution in microgravity.[88,93]

Fluid loss, decreased plasma volume, increased osmolality, diminished fluid intake and decreased urine volume (with negative fluid balance), increased renal excretion of electrolytes (Ca, P, Na, K, Cl), and negative nitrogen balance all have been documented during spaceflight[17,93] (see also Chapter 4, this volume). Elevated concentrations of ionized calcium in the blood, which are thought to be present during spaceflight, could reflect reductions in blood albumin, changes in blood pH, elevated release of calcium from bone, dehydration, or renal problems.[93,97] Negative calcium balance can arise from hypercalcemia, hypercalciuria, malabsorption through the intestine, resorption at the renal tubules, and other causes.[17,35,93] Evidence that calcium regulation is involved in the changes seen in fluid-electrolyte balance associated with fluid redistribution[88,97] could help to clarify the homeostatic shifts in calcium and its distribution in microgravity.

One negative consequence of disrupted calcium metabolism in space is the increased risk of forming kidney stones.[98] This risk seems to be related to the disruption of bone-blood calcium homeostasis, the metabolic shifts associated with fluid redistribution, emotional and physical stress, and insufficient or inappropriate exercise or rehydration during flight. Indicators suggesting increased stone-forming potential noted in humans include hypercalciuria, decreased urine volume, increased concentrations of oxalates and uric acid in the urine, decreased urinary pH, and decreases in citrate and magnesium concentrations in urine.[17,93]

Animal studies aboard biosatellites have demonstrated reductions in somatotropic and thyrotropic activity of the anterior pituitary gland, as expressed in decreased production of growth and thyroid hormones; other findings include decreased blood concentrations of PTH and calcitonin.[99] These data are difficult to extrapolate to humans, however. The headward redistribution of body fluids characteristic of humans takes place to a much lesser extent in animals. Moreover, rats flown in space showed less severe stress reactions, e.g., catecholamines were low, and blood ACTH and corticosterone were negatively correlated with each other.[99]

Another potential modulator of calcium homeostasis in humans is the immune system. Blood concentrations of osteoclast activating factor (OAF) have been elevated in bed-rest subjects[100]; however, meaningful interpretation of these data await assessments of interrelated cytokines, e.g., interleukin-1[101] and others perhaps yet to be identified.

Localized, short-acting cytokines are thought to play significant roles in bone changes in microgravity as well.[102] Evidence for this supposition includes the widespread, heterogeneous changes revealed by histomorphometry, densitometry, and biomechanical function in different bones. Mechanical stimulation, thought to be the chief inductor of bone-cell activity, is transformed into metabolic signals through yet-undefined mechanisms, which may include electrical potentials created as a result of cell or cell-wall deformation, streaming

potentials, or the effects of mechanoreceptors (the integrins).[103] These processes are regulated mostly at the local (tissue) level.[92]

Further evidence for the influence of local factors comes from the demonstrated sensitivity of bone-cell cultures flown on the 13-day Kosmos-2229 mission.[104] Osteocalcin gene expression also was decreased in the periosteum of the long bones of rats after a 4-day Shuttle flight, as well as in a control group in which the sciatic nerve had been severed on one side.[105] Another study involving the latter technique revealed a decrease in number of osteoblast precursor cells in a culture of femoral stromal cells after 11 days.[106] Proliferative activity did not change, but differentiation of osteoblasts was significantly attenuated, as was their capacity to form mineralization loci. This latter finding resembles previous in vivo findings of inhibited progressions of osteoprogenitor cells into preosteoblasts[58] and osteogenic differentiation of osteoblasts.[46] The precise means by which bone formation is suppressed in response to mechanical unloading are as yet unclear, and require further study.[8,9,92]

V. Bone Biomechanics and the Prediction of Osteopenia

Bone possesses a relatively high "strength reserve"; for example, the breaking loads of the vertebral trabeculae and tibial compact bone are 10 times the corresponding physiological loads.[11] The main reason for declines in the mechanical strength of bones is loss of bone mass. Thus, fracture risk in humans can be predicted noninvasively by measuring changes in mineral density and identifying them with changes in bone mass.[13] However, the function relating the mechanical properties of bone with mineral saturation is exponential, particularly for trabecular bone.[10] Therefore slight changes in mineral density can be associated with significant shifts in the strength of trabecular bone. For example, bone strength in rats can diminish after as little as 1 or 2 weeks of spaceflight, but need not be accompanied by changes in mineral saturation.[36,42]

Loss of bone mass thus cannot be the only cause of decreases in bone strength. As an example, only 75% of the decrease in load-carrying capacity of bones can be attributed to age-related losses of bone mass.[107] (Strength losses, which exceed the reductions in mineral density, are particularly apparent in age-related changes of the vertebrae and femoral neck.[108,109]) Organic-matrix composition, bone porosity, orientation of collagen fibers in osteons, the nature of the "collagen-crystal" bond,[10,36,42,49,110] and other yet-unknown aspects of bone composition and microstructure[35] are important to the mechanical status of bone.

Animal experiments have confirmed that many of these factors are accompanied by decreases in bone.[10,36,49] In humans, the age-related decrease in the strength of the lumbar vertebrae is influenced not only by thinning of the trabeculae, but by loss of the continuous network therein.[111] As discussed earlier, subjects in a 120-day head-down bed-rest study showed histomorphometric evidence of trabecular restructuring in the iliac crest (decreased number and increased thickness of trabeculae) without having lost bone mass.[90] Biomechanical tests of the biopsy specimens after 370 days of head-down bed rest revealed signs of decreased resistance to deformation and diminished strength resulting from increased brittleness, both of which are typical of aging bone.[91]

The rate of onset and the severity of osteopenia depend on several factors,[65,112] including hereditary factors (gender, age, body weight, physical fitness, metabolic rate, presence of hereditary diseases) environmental factors (nature and type of motor activity, nutritional status, and use of drugs such as steroids, heparin, anticonvulsants, or antacids), and postnatal development history.[113] No tests exist at present that can account for all of these factors in estimating the risk of fracture or osteopenia. In one study of 94 women (40–80 years of age) who had osteoporosis with compound vertebral fractures, quantitative computer tomography revealed that only 21% had mineral density that was less than age-appropriate values.[114] These data do not provide a specific density threshold for fracture, but rather suggest that the probability of fracture increases linearly. This was particularly true of the vertebrae, for which density decreased from 110 to 60 mg/cm^3.[114–116] Another study that involved dual-photon absorptiometry revealed that 50% of the women with postmenopausal osteoporosis (identified through the presence of fractures on X-rays) had normal bone mass for their ages.[13]

Many authors believe that spontaneous bone fractures require a minimum 30–40% loss of mineral density.[65,66,114] Species differences in this amount undoubtedly are determined genetically; however, subpopulations within species might be distinguishable on the basis of rate of bone loss during postnatal development.[117] Nevertheless, fracture clearly involves bone strength as well as the force applied to that bone, and thus fractures can occur when the force applied exceeds the tolerance of the bone. The clinical and epidemiological literature on the use of repeated densitometry to predict fracture risk (e.g., Ref. 117) could be supplemented with assessments of biochemical indicators of bone turnover (e.g., urinary hydroxyproline and pyridinoline, blood alkaline phosphatase and osteocalcin) to provide another means of predicting who is likely to develop osteopenia or fractures.

The observed mean monthly loss of 1–3% of the bone mineral in the spine and femoral neck suggests that the probability of spontaneous fractures in cosmonauts after year-long spaceflights would not be great. However, this statement may be premature. First, individual variation may reach 2–3 times this mean; second, sclerotic increases in mineral density in the vertebrae may be associated with *decreased* mechanical strength. The crystallinity of the mineral component increases with age[118]; moreover, the increased mineral density noted in some patients with systemic[27] or senile[119] osteoporosis is suggestive of increased brittleness. Although some evidence exists to suggest that genetic characteristics of cultured bone cells may be a way to identify hereditary contributions to osteoporosis,[120] as of this writing, precise predictions of bone pathology arising from changes in weight loads on the skeleton are not yet possible.

VI. Countering Bone Loss in Weightlessness

The effectiveness of current countermeasures in maintaining local mineral density in cosmonauts and bed-rest subjects is illustrated in Fig. 11. Various exercises (e.g., running on a weighted treadmill, isometric exercises, etc.) can suppress osteopenia after spaceflight or head-down bed rest. However, these results also could reflect differences among individuals. In any event, exercise clearly is important in protecting the status of muscles and motor functions in space crews.[121]

The existing exercise equipment aboard spacecraft seems to provide mechanical loads inadequate to compensate for the loss of loading experienced in 1 *g*.[122] The 370-day bed-rest study demonstrated that a countermeasure protocol that includes exercise, drugs, and dietary supplements can significantly diminish negative calcium balance,[73,74] and can stabilize mineral-density loss in some bones (heel, tibia, femoral neck). However, in another study, exercise had positive effects only in combination with drugs.[69]

Nevertheless, physical exercise should be considered an essential means of preventing bone mass loss and maintaining muscular and aerobic physical conditioning during spaceflight. Isokinetic and isometric exercise[121] and exercise accompanied by isotonic contractions[123] are thought to maintain muscle strength and aerobic capacity (90–100% O_2 consumption) in astronauts. Exercise involving eccentric muscle contractions may be still more effective in maintaining muscle strength and endurance.

Preventing osteopenia in space crews, in our opinion, requires a system that can provide mechanical loads to each area of the body. Also, countermeasure programs must be adaptable for different individuals, and must minimize the time needed during flight without jeopardizing the health of space crews.

VII. Summary and Issues for Future Research

Living bone undergoes a complex set of changes in microgravity as a result of the elimination of weight loading on the skeleton. Whether in spaceflight or in prolonged bed rest, mineral density and bone mass undergo characteristic changes that are related to the position of the bone with respect to the gravitational vector. In the lower body, the legs, pelvis, and lumbar vertebrae show consistent patterns of decrease in mineral density. In contrast, bone density in the upper body (ribs, thoracic vertebrae, arms, or skull) tends to not change, or occasionally increases. Selective hypermineralization of the lumbar-vertebral bodies, accompanied by unchanged or diminished mineral density of these bodies, has been detected in about one third of the cosmonauts or bed-rest subjects tested.

Establishing reliable correlations between mineral density and flight duration has not been possible; however, no evidence exists to suggest that loss of bone mineral is self-limiting in space. However, head-down bed-rest studies have shown that the demineralization process, particularly in the femoral neck, can be stabilized—or even reversed—through the use of carefully controlled countermeasures.

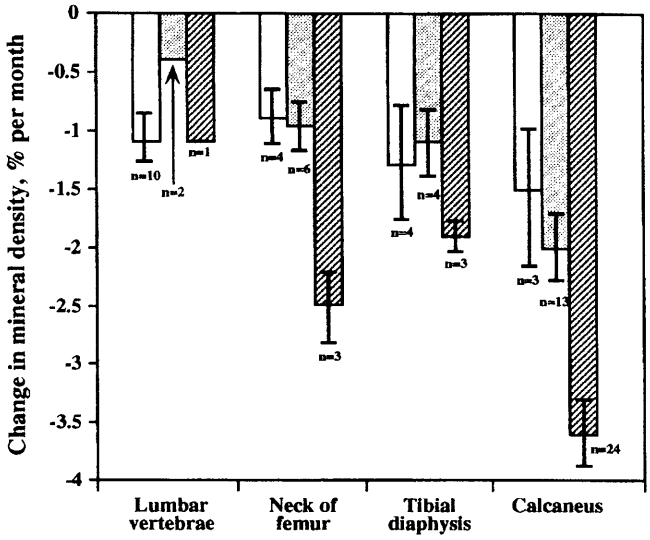

Fig. 11 Changes in bone mineral density after 4.5- to 6-month spaceflight (white bars) or 6- to 12-month head-down bed rest. Shaded bars, bed rest with countermeasures; hatched bars, bed rest with no countermeasures. From Refs. 10, 28, 69, 74, and 78.

Loss of bone mass leads to diminished bone strength. The changes in bone biomechanics in space, evident before mineral loss becomes statistically significant, probably increase the likelihood of damage from trauma. Morphologic changes in iliac-crest biopsy samples were evident after as few as 7 days of bed rest. However, morphological changes did not always coincide with biomechanical assessments of the same samples, or with densitometric assessments of skeletal segments close to those studied biomechanically.

The nature of the changes described above suggests that one reason for loss of bone mass in space is a deficit or change in the nature of mechanical loading, as evidenced by depressed osteogenesis and retardation of bone formation and biomineralization. Activation of resorption probably plays a significant role in the development of negative calcium balance and bone demineralization in weightlessness. However, some investigators have proposed that the primary reaction of bone to weightlessness is the disruption of the association of the matrix and the mineral component (the "collagen-crystal" bond).[10,35,36] Indeed, when mechanical stimulation is absent, the bone "demand" for calcium may decrease, leading to increases in plasma calcium concentration.[10,35] The ensuing decrease in blood PTH could, either directly or through modulating vitamin D synthesis, provoke decreased resorption of calcium in the renal tubules and reduced absorption in the intestine.[35] However, the data that could be considered sufficient to justify the role of catabolic processes are based on pathologic conditions on Earth.

Another potential explanation for regional bone loss in space may be a reduction in resorptive processes and bone remodeling normally triggered by microtraumas associated with nor-

mal standing and walking on Earth. An overall retardation in bone restructuring could be accompanied by areas of hypermineralization. If this is so, then the further decreases in bone strength noted during readaptation may reflect acceleration of remodeling and activation of resorption in response to mechanical loading. Thus, the skeletal changes noted in weightlessness, particularly those in the lower body, could be considered a kind of "local osteopenia" occurring in response to deficient mechanical loading in space.

Densitometry has revealed relatively small losses of bone mass after spaceflight—mean 1% per month. Although the mechanical reserves of the skeleton suggest that the risk of spontaneous bone fracture is not great after 1-year spaceflights, individual variations are substantial, and mineral density may not reflect the mechanical strength of the bone. Since no data are available during spaceflight, and since experiencing impact loads during landing is a distinct possibility, the risk of damaging injury-prone skeletal areas (e.g., vertebral bodies) under these conditions cannot be ruled out.

Data on the recovery of bone mass in cosmonauts are inadequate and ambiguous; estimates of the rate and completeness of recovery will be subject to individual variation. Different skeletal areas may recover at different rates, or not at all. If bone lost during spaceflight cannot be replaced, then age-dependent osteoporosis may appear earlier than otherwise expected. Individuals with particularly high rates of bone turnover during flight may run the risk of calcification in soft tissue or blood vessels.

In our opinion, preventing or ameliorating osteopenia and other adverse consequences can be achieved only through the use of a combination of interventions, one essential component of which must be exercise. An ideal system would provide 1-*g*-like loads to the muscles and bones of the spine, pelvic girdle, and thighs, and would probably involve the use of drugs that slow bone resorption, e.g., biphosphonates. Nutritional and metabolic means also can be used to reduce the risk of kidney-stone formation.

In conclusion, we propose that the following tasks represent crucial issues for sustaining the human presence in space:

- Identifying the mechanisms that cause osteopenia in specific areas of the skeleton so that likely bone traumas can be avoided.
- Establishing the rate of bone loss and the time needed to attain new, steady-state bone-strength threshold values in order to predict the risks of traumatic or nontraumatic fractures.
- Investigating the reversibility of bone loss and bone-strength thresholds with respect to their lifetime consequences.
- Elucidating the nature of and reasons for local hypermineralization of trabecular bone in the vertebrae.
- Clarifying and distinguishing among the relative roles of systemic and local factors in regulating calcium metabolism in bone and in the entire body.
- Calculating the probability of developing kidney stones and soft-tissue calcification resulting from disrupted calcium homeostasis in weightlessness.
- Identifying means with which these events can be predicted, diagnosed, and treated early.
- Developing countermeasures that will prevent or ameliorate the changes described while they are developing.

We believe that resolving these issues will hasten understanding of bone adaptation during spaceflight and other forms of gravitational unloading. Other potential Earth benefits may well include fostering the development of new diagnostic methods with which to predict the risk of fractures and osteopenia, as well as developing means of preventing or correcting prepathologic conditions early, before irreversible damage can take place.

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